Improved Regeneration of Shoots from Garlic Callus

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Plantlet regeneration from garlic (*Allium sativum* L.) callus has been suggested as a source of somaclonal variants (Nagasawa and Finer, 1988; Novak et al., 1986), a means of freeing plants from endemic viruses (Abo El-Nil, 1977; Koch and Salomon, 1994), and a target for transformation (Myers and Simon, 1993). However, slow callus growth and poor regeneration limits the use of these techniques.

Several medium additives, including plant growth regulators and N, affect regeneration from callus in other *Allium* species (Dunstan and Short, 1977). Our study tested the effect of several medium additives on the rate of garlic callus growth and shoot regeneration.

One hundred cloves from 10 randomly chosen 'Frankon' garlic bulbs of a uniform lot were peeled, soaked for 1 h in 3% NaOCl, and then rinsed 45 min in three changes of sterile water. The shoot basal plate from a clove plus 1 to 2 mm of the leaf bases (scales) were excised, cut into two to three explants, and placed (one clove per dish) in plastic petri dishes (50 mm in diameter) with 10 ml of callus induction medium. This medium was BDS-based (Dunstan and Short, 1977) with 3 μ M 2,4-dichlorophenoxyacetic acid (2,4-D), 3 μ M 1*H*-indole-3-acetic acid (IAA), 3% sucrose, and 0.3% Phytagel (Sigma Chemical Co., St. Louis), adjusted to pH 5.7 before being autoclaved for 20 min at 121°C. Cultures were incubated at 25°C in darkness for 6 weeks, after which the callus was transferred to fresh callus induction medium for another 6 weeks before being used to compare nine additive combinations.

These additive combinations included the callus induction medium previously described; five variations of this medium by adding 9 μ M of the cytokinins 6-benzyladenine (BA) or Δ^2 iso-pentenyladenine (2iP), inorganic N (2.53 g additional KNO₃/liter), organic N (900 mg casein hydrolysate/liter), or 60 g additional sucrose/liter and three variations containing alternative combinations of auxin and cytokinins: 5 or 10 μ M BA, 10 or 20 μ M IAA, and/or 3 μ M 4-amino-3,5,6-trichloropicolinic acid (picloram). The BDS medium was prepared as previously described; IAA and 2iP were added after autoclaving.

Callus was divided into portions of 270 $\pm$ 10 mg fresh weight and placed one portion per plastic petri dish (50 mm in diameter) containing 10 ml medium. Fifteen cultures were established for each medium. Cultures were incubated at 25°C in darkness for 6 weeks. Callus of each culture then was weighed, transferred to plastic petri dishes (90 mm in diameter) with 40 ml fresh medium, and incubated as previously described. Final fresh weight of callus was assessed 12 weeks after establishment. Calluses from each medium then were transferred to twenty 100-ml glass jars with plastic lids filled with 30 ml BDS-based regeneration medium (25 μ M kinetin and 10 μ M IAA) and incubated at 25°C with a 16-h photo-

period. The number of regenerated shoots was counted 8 weeks later. Effects of medium supplements on callus weight and number of shoots were compared by analysis of variance; treatments were compared by Duncan's multiple range test.

Callus growth on the standard medium was equal to that in all other media, with the exception of the medium with extra KNO₃ (Table 1). Callus grew $\approx$ 33% faster on this medium and was friable; callus grown with organic N did not show improved growth.

Shoot regeneration was best from compact callus grown on the medium with 2,4-D, IAA, and 2iP (Table 1). This additive combination gave a 42.5% increase in shoot regeneration over calluses grown on similar medium but without 2iP or with BA instead of 2iP. The rapidly growing calluses from medium with additional KNO₃ had a low regeneration rate.

This improvement in regeneration rate will help develop systems to increase variation in this sterile species.

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Table 1. Garlic callus weight after 6 and 12 weeks of culture (25°C, in darkness) on nine BDS-based callus growth media (Duncan and Short, 1977) and the number of shoots regenerated from cultures following transfer to BDS-based regeneration medium (25 μ M kinetin, 10 μ M IAA) and incubation at 25°C with a 16-h photoperiod.

Medium additives	Mean fresh wt (g)			Mean no. shoots/g callus
	Culture (wks)			
	0	6	12	
3 μ M 2,4-D, 3 μ M IAA	0.27	2.65 b ^a	7.59 b	0.41 b
3 μ M 2,4-D, 3 μ M IAA, 9 μ M BA	0.26	2.47 b	7.07 b	0.40 b
3 μ M 2,4-D, 3 μ M IAA, 9 μ M 2iP	0.27	2.26 b	7.93 b	0.57 a
3 μ M 2,4-D, 3 μ M IAA, 9% sucrose	0.27	1.43 c	3.26 c	0.40 b
3 μ M 2,4-D, 3 μ M IAA, 900 mg casein hydrolysate/liter	0.27	2.33 b	6.64 b	0.48 ab
3 μ M 2,4-D, 3 μ M IAA, 2.5 g extra KNO ₃ /liter	0.26	3.50 a	12.04 a	0.13 c
5 μ M BAP, 3 μ M picloram	0.28	2.50 b	7.23 b	0.40 b
5 μ M BAP, 10 μ M IAA	0.28	2.40 b	3.54 c	0.37 b
10 μ M BAP, 20 μ M IAA	0.27	2.18 b	4.84 c	0.37 b
Mean	0.27	2.41	6.68	0.39

^aMean separation within a column by Duncan's multiple range test at $P \leq 0.05$.

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